



Article

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PHOTOSYNTHETIC POTENTIAL AND PRODUCTIVITY OF COMMON BEANS UNDER HERBICIDE EFFECT

Potencial Fotossintético e Produtividade do Feijoeiro Comum sob Efeito de Herbicidas

ABSTRACT - The objective of this study was to evaluate the effects of the application of contact herbicides recommended for common bean crops, as for chlorophyll *a* fluorescence parameters, leaf soluble proteins content (LSPC) and productivity. The experiment was conducted on the field with five treatments, which were the application of the following herbicides: bentazon (720 g ha⁻¹), fluazifop-p-butil (187.5 g ha⁻¹), fomesafen (250 g ha⁻¹) fluazifop-p-butil + fomesafen (187.5 + 250 g ha⁻¹), and a manually weeded control treatment without herbicide application, in a randomized block design with four replications. Bentazon was the only herbicide causing significant reductions, but only until the first day after herbicide application (DAA), on the following chlorophyll *a* fluorescence parameters: maximum quantum yield of photosystem II (F_v/F_m), effective quantum yield of photosystem II (Φ_{PSII}), photochemical quenching (qP); it also induced an increase in non-photochemical quenching (NPQ). F_v/F_m was the best parameter to indicate herbicide effect on the photosynthetic apparatus of plants in the field. Chlorophyll *a* fluorescence parameters obtained in light-adapted leaves underwent a high environmental influence, especially deriving from the variation in the photosynthetic photon flow density (PPFD) during measurements; they are not recommended to evaluate the effects of herbicides on the field. None of the applied herbicides evaluated caused reductions in grain yield; therefore, they are recommended for common bean crops.

Keywords: *Phaseolus vulgaris* L., chlorophyll *a* fluorescence, photosystems, photoinhibition.

RESUMO - Objetivou-se neste estudo avaliar os efeitos da aplicação de herbicidas pós-emergentes, recomendados para a cultura do feijoeiro comum, nas variáveis de fluorescência da clorofila *a*, no conteúdo de proteína solúvel foliar (LSPC) e na produtividade de grãos. O experimento foi conduzido em campo e teve cinco tratamentos, que constaram da aplicação dos seguintes herbicidas: bentazon (720 g ha⁻¹), fluazifop-p-butil (187,5 g ha⁻¹), fomesafen (250 g ha⁻¹), fluazifop-p-butil + fomesafen (187,5 + 250 g ha⁻¹), além de um tratamento controle capinado, sem aplicação de herbicida, com delineamento de blocos ao acaso com quatro repetições. O bentazon foi o único herbicida que causou reduções significativas, mas somente até o primeiro dia após aplicação (DAA), nas seguintes variáveis de fluorescência da clorofila *a*: rendimento quântico máximo do fotossistema II (F_v/F_m), rendimento quântico efetivo do fotossistema II (Φ_{FSII}), bem como no coeficiente de extinção fotoquímica (qP), e promoveu incremento na variável de coeficiente de extinção não fotoquímica (NPQ). Entre elas, o F_v/F_m foi a variável mais indicadora dos efeitos decorrentes da aplicação de herbicidas no aparato fotossintético de plantas no campo. As variáveis de fluorescência da clorofila *a*

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obtidas em folhas adaptadas à luz sofreram grande influência do ambiente, principalmente da variação da densidade de fluxo de fótons fotossintéticos (DFFF) durante as medidas, não sendo recomendadas como indicadoras do efeito dos herbicidas no campo. Nenhum dos herbicidas avaliados causou redução na produtividade, sendo recomendáveis para a cultura do feijoeiro comum.

Palavras-chave: *Phaseolus vulgaris* L., fluorescência da clorofila *a*, fotossistemas, fotoinibição.

INTRODUCTION

Common beans (*Phaseolus vulgaris*) have a superficial root system and a slow initial growth; they are very sensitive to environmental stresses and to competition with weeds (Teixeira et al., 2009; Borchardt et al., 2011). Its culture has a relatively short cycle, and is susceptible to weed interference processes, especially at the early stages of its development (Cobucci et al., 1999; Costa et al., 2013).

The use and correct management of herbicides allows controlling weed infestation with practicality, allowing better grain yield, especially in large cultivation areas (Machado et al., 2006). The adoption of chemical weed control in bean crops, besides its practicality and effectiveness, is of fundamental importance, mainly in order to avoid great losses caused by the competition with weeds, which can cause productivity reductions up to 80% (Parreira et al., 2014).

However, the application of herbicides may cause crop phytotoxicity; even those presenting selectivity can cause damages, and this may be verified visually or even through changes in the physiological variables of plants (Fedtke and Schmidt, 2013; Langaro et al., 2016). Most herbicide molecules act primarily at specific sites as inhibitors of enzymes involved in the main metabolic pathways of plants, and present high specific affinity for their respective sites of action (Dayan et al., 2010). Among these affected metabolic pathways, there is energy transfer in photosystems, to be used in the photosynthesis of plant cells (Dayan and Duke, 2014). After being absorbed and acting at their respective primary sites of action, herbicides can cause a series of biochemical and physiological events that are harmful to plants (Devine et al., 1993), such as the intensification of the photoinhibition process, for example (Roháček, 2008).

Photosynthetic organisms, when subjected to high photosynthetic photon flux densities (PPFD) associated with the photosynthesis reduction caused by an environmental or chemical stress, such as herbicides, stimulate the production of reactive oxygen species (ROS). The production of ROS is obtained from the electrons released in the photolysis of water, because the transport of photosystem electrons is reduced by the environmental stress. However, these ROS cause oxidative stress, which can lead to the integrity loss of membranes such as thylakoids, where photosystems are, and which may stop being functional, depending on the intensity of the stress (Baker, 2008). ROS are also intracellular markers that indicate a stressful situation, which contribute to the activation of energy dissipation mechanisms in photosystems, so as to avoid photoinhibition (Zivcak et al., 2015).

Plants have some protective mechanisms to prevent severe damages to the photosynthetic apparatus, which include repairing and replacing D₁ proteins in the Reaction Centers of Photosystem II (PSII), until dissipation of excess light energy (Baker, 2008), as in the xanthophyll cycle, which seems to be one of the most important mechanisms of PSII excess energy dissipation, in the form of heat. In addition to these, other processes, such as the pseudocyclic photophosphorylation, the ascorbate glutathione cycle and the photorespiration, contribute to reduce the photoinhibitory effect, by the consumption of the NADPH₂ produced in photosystems (Pimentel, 2014). In the xanthophyll cycle, violaxanthin is epoxidized and reduced to zeaxanthin during the day under high PPFD and stresses, consuming part of the excess electrons in the photosystem, and at night, with a lower metabolism, zeaxanthin is gradually de-epoxidized to violaxanthin, thus reducing the photoinhibitory effect (Takahashi and Badger, 2011).

The analysis of the variables obtained by the emission of chlorophyll *a* fluorescence is already a well known technique, and is used in several work areas to discriminate between the effects of stress (Pimentel, 2014). Studies conducted to better understand the relationship between

these chlorophyll *a* fluorescence emission variables and the photosynthetic potential associated with the physiological conditions of the plant under study became frequent, due to the development and commercial availability of fast and low cost portable fluorometers, compared to other photosynthetic activity measuring equipments, which helped the use of this methodology in evaluations and in the selection of a large number of plants (Murchie and Lawson, 2013).

Several studies have been conducted in order to evaluate the influence of biotic and abiotic stresses on these variables, such as the phytotoxicity caused by herbicides (Zhang et al., 2015), by the action of phytopathogens (Costa et al., 2009), under stress conditions due to water deficit (Lima et al., 2002), salinity or temperatures above or below the ideal one for the plant under study (Zanandrea et al., 2006), influence of shading and competition among plants, that is, a series of stress situations to which plants can be subjected (Murchie and Lawson, 2013).

Therefore, this study aimed at evaluating the effects of different treatments with herbicides on the variables of chlorophyll *a* fluorescence emission, on the leaf soluble protein content (LSPC), which is proportional to the activity of Ribulose Bisphosphate Carboxylase/Oxygenase (Rubisco), according to Pimentel (2006), and on common bean productivity, considering its indication as a variable for plant selection.

MATERIAL AND METHODS

The experiment was installed on the field (22°45' S and 43°41' W), and the climate of the region is Aw-type, according to Köppen's classification, with hot and rainy summer and dry winter. The soil type of the experimental area is a Red-Yellow Argisol, and its chemical analysis showed the following characteristics: pH in water 5.4; 2.3 cmol_c dm⁻³ of Ca; 1.0 cmol_c dm⁻³ of Mg; 0.0 cmol_c dm⁻³ of Al; 1.5 cmol_c dm⁻³ of H⁺ Al; 4.7 mg dm⁻³ of available P; 135 mg dm⁻³ of available K; and 71% base saturation (V%). Total precipitation, reference evapotranspiration, and average maximum and minimum temperatures were 114 mm, 189.8 mm, 28.4 °C and 18.3 °C, respectively. The experimental period comprised the months from April to July 2015. Before the implantation of the crop, the soil was prepared; this consisted of a plowing and light harrowing, and then, in the mechanical opening of planting furrows. The Carioca common bean cultivar was used in the crop, obtained by the Agronomic Institute of Campinas (Instituto Agrônômico de Campinas – IAC) and with a large number of studies in literature (Vieira et al., 2006). Plots were composed of five 5 m long lines, spaced 0.5 m apart, totaling an area of 10 m² per plot. After germination, 12 plants per meter were obtained, totalizing a stand of 240,000 ha⁻¹ plants. Planting fertilization was applied using 20 kg ha⁻¹ of N, 90 kg ha⁻¹ of P₂O₅ and 20 kg ha⁻¹ of K₂O; top-dressing was performed 25 days after sowing (DAS) with 40 kg ha⁻¹ of N, according to the recommendations of Vieira et al. (2006).

The five treatments consisted of post-emergence applications, at 23 DAS, of the following herbicides: bentazon (720 g ha⁻¹), fluazifop-p-butyl (187.5 g ha⁻¹), fomesafen (250 g ha⁻¹), fluazifop-p-butyl + fomesafen (187.5 + 250 g ha⁻¹) and a fifth weed control treatment without herbicide application. A randomized block design with five treatments and four replications was used. In order to apply the herbicides, a CO₂ pressurized backpack sprayer was used, equipped with a bar with four TT 110.02 fan-type nozzles, operating at the constant pressure of 20 PSI and applying a spray volume of 192 L ha⁻¹.

On the day of spraying and on the following six days, as well as 14, 21 and 28 days after application (DAA), chlorophyll *a* fluorescence variables were evaluated in order to monitor the use of light energy by the photosynthetic apparatus of plants under the effect of the applied herbicides. Chlorophyll *a* fluorescence analyses were performed with a modulated fluorimeter (MINI-PAM model, by Walz, Germany), and measurement times were 5 am (before dawn), 10 am and 7 pm (evening). Measurements on leaves adapted to the dark were the ones made at 5 am and 7 pm, as proposed by Pimentel et al. (2005). Moreover, the difference between these values was also calculated, in order to analyze the photoinhibition intensity occurred throughout the day (measured at 7 pm, minus the one measured at 5 am on the same day), and the recovery capacity over night (measured at 5 am of one day, minus the one measured at 7 pm the previous day). On the other hand, measurements on leaves adapted to light were made at 10 am, when photosynthesis is high (Pimentel, 2006). Therefore, the maximum (F_m) and minimum (F_o)

fluorescence were measured in leaves adapted to the dark (5 am and 7 pm). F_0 was measured on leaves after their adaptation to the dark, for at least 30 minutes, under low and modulated illumination ($<0.5 \mu\text{mol m}^{-2} \text{s}^{-1}$), and F_m was measured after a pulse of light saturation ($18,000 \mu\text{mol m}^{-2} \text{s}^{-1}$) lasting 3 s; from these measurements, the yield of the fluorescence variable was calculated ($F_v = F_m - F_0$), as described by Schreiber et al. (1994). Stationary (F_s), maximum (F_m') and minimum (F_0') fluorescence were measured on leaves adapted to light, under a $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ actinic illumination, and variable fluorescence was also calculated ($\Delta F = F_m' - F_s$). From these measurements, the calculated chlorophyll *a* fluorescence variables were: the maximum quantum yield of photosystem II (PS II) ($F_v/F_m = (F_m - F_0)/F_m$); the effective quantum yield of PS II ($\Phi_{\text{PSII}} = F_m' - F_t/F_m'$); the photochemical extinction coefficient ($qP = (F_m' - F_t)/(F_m' - F_0')$); and the non-photochemical extinction coefficient ($\text{NPQ} = (F_m - F_m')/F_m'$) as described by Maxwell and Johnson (2000) and Roháček et al. (2008). The amplitudes of PPFD variation on the days of chlorophyll *a* fluorescence analysis were: 280-450, 450-1009, 190-660, 1125-1508, 1220-1475, 635-779, 282-1469, 1150-1330 and 1100-1330 $\mu\text{mol m}^{-2} \text{s}^{-1}$, for 0, 1, 2, 3, 4, 5, 6, 14 and 21 DAA, respectively, and the temperatures at the start time of reading on leaves adapted to light (10 am) were: 19.9; 19.2; 17.7; 19.6; 22.4; 22.6; 22.1; 20.9; and 22.2 °C for 0, 1, 2, 3, 4, 5, 6, 14 and 21 DAA, respectively.

After determining the fluorescence variables of chlorophyll *a*, measured at 10 am, leaf samples were collected for LSPC quantification. The collected leaves were the same as those used for fluorescence determination. The collection was performed on day zero, two, five, seven, 14, 21 and 28 DAA. One of the lateral leaflets of the youngest fully expanded leaf of three plants was collected in each plot. Collected samples were immediately wrapped in aluminum foil and immersed in liquid nitrogen; they were taken to the laboratory for colorimetric measurements in a spectrophotometer (Spectronic 20 model, by Milton Roy, USA). In these samples, LSPC was determined as mg of soluble protein/fresh matter gram, according to the methodology proposed by Bradford (1976).

Finally, at physiological maturation, all plants from the two central lines of each plot were collected, discarding 0.5 m of each end so as to determine production components: number of pods per plant, number of grains per pod, 100-grain weight and grain yield.

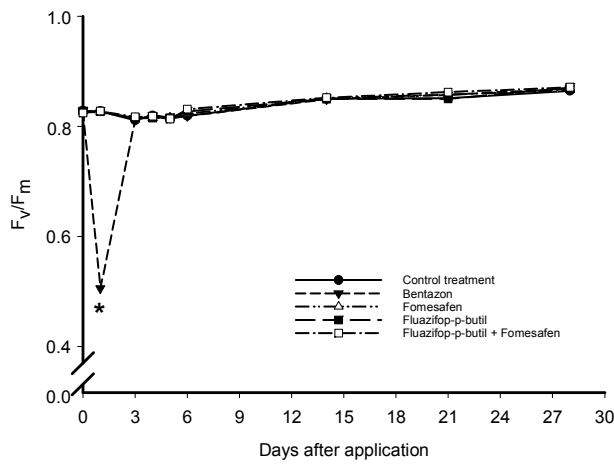
The obtained data were submitted to analysis of variance and, when significance was detected between treatments, means were compared by Student-Newman-Keuls test for chlorophyll *a* fluorescence variables and by Tukey's test for LSPC and productivity ($p \leq 0.05$).

RESULTS AND DISCUSSION

The first evaluations on the F_v/F_m variable on leaves adapted to the dark were made at dawn (5 am), at 0 DAA (Figure 1), before the application of the herbicides, and there were no significant variations in the F_v/F_m values measured for the treatments; the mean value obtained between them was 0.827, which is considered an adequate value for a plant without stress (Maxwell and Johnson, 2000). In the analyses performed on leaves adapted to the dark, the plant is photochemically inactive, and the photosystem components present their maximum potential to absorb photons and to produce ATP and NADPH_2 for the assimilation of CO_2 , given by F_v/F_m (Roháček et al., 2008).

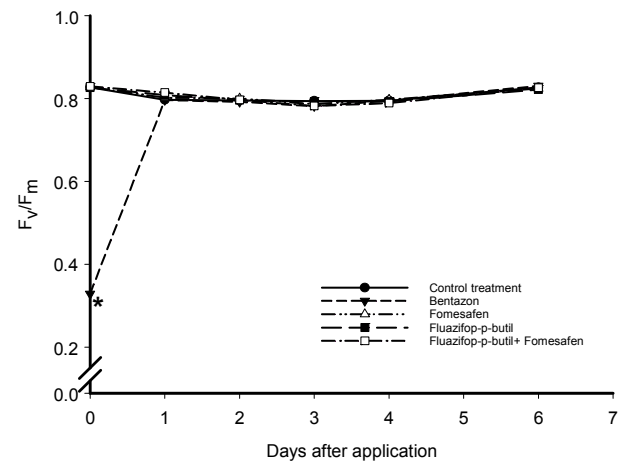
With the application of herbicides, at 0 DAA, only bentazon caused a significant F_v/F_m decrease, in relation to all the other treatments, soon after the application of the products; this was observed in measurements made at night (7 pm) at 0 DAA (Figure 2), and at dawn on the following day from the application (1 DAA) (Figure 1). However, in the night period of the day after application (Figure 2), there were no further differences among treatments; this persisted in all subsequent evaluations, which were performed up to 6 DAA (Figure 2). As for the F_v/F_m readings at dawn, they were maintained until 28 DAA, considering that the F_0 value obtained at dawn is necessary to calculate the chlorophyll *a* fluorescence variables of plants adapted to light. In addition, the F_v/F_m dawn readings were not performed at 2 DAA, due to the occurrence of rainfall. The same happened for the F_v/F_m night readings at 5 DAA.

Mahoney and Penner (1975) claim that the rapid metabolism of bentazon in bean trifoliates functions as a mechanism of selectivity to the herbicide. In tolerant species, the product is



The asterisk represents the statistical difference ($p \leq 0.05$) on the evaluation day.

Figure 1 - Maximum quantum yield values of photosystem II (F_v/F_m) in the treatments, measured at dawn (5 am).



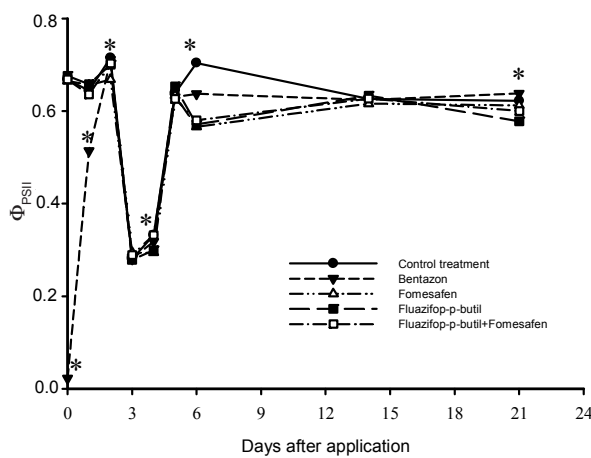
The asterisk represents the statistical difference ($p \leq 0.05$) on the evaluation day.

Figure 2 - Maximum quantum yield values of photosystem II (F_v/F_m) of the treatments, measured at night (7 pm).

metabolized by the formation of hydroxybenzazepine, with disruption of the heterocyclic ring (Cobucci and Machado, 1999). The F_v/F_m value results in this study, from 1 DAA onwards, are within the 0.75 to 0.85 range, which is considered an adequate value for a stress-free plant, as mentioned (Schreiber et al., 1994).

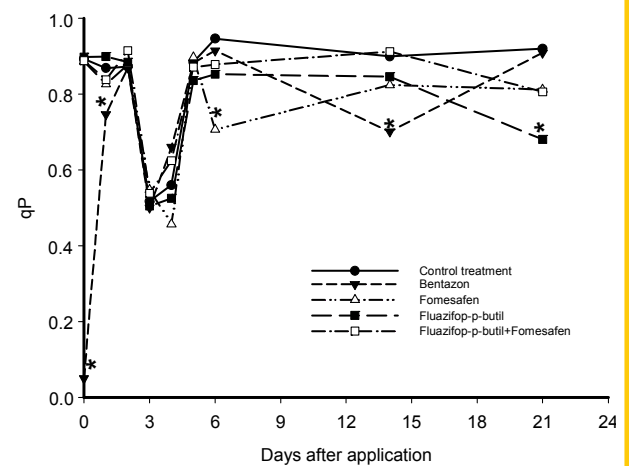
As for the chlorophyll *a* fluorescence variables obtained in light-adapted leaves, which were measured at 10 am, the application of bentazon was also the only treatment promoting Φ_{PSII} (Figure 3) and *qP* (Figure 4) decline. The values measured for these variables were close to zero in measurements made after applying the herbicides, at 0 DAA. The reduction also continued at 1 DAA, but returning to the values of the other treatments from the second DAA (Figures 3 and 4).

The reduction of these two variables, Φ_{PSII} and *qP*, demonstrates a rapid effect of this herbicide on the photosynthetic activity of plants, but this was reversed at 2 DAA. During the days following the application, there was an oscillation in Φ_{PSII} and *qP* values (Figures 3 and 4), probably due to variations mainly of PPFD, which was greatly reduced at 3 DAA, increasing during the following



The asterisk represents the statistical difference ($p \leq 0.05$) on the evaluation day.

Figure 3 - Effective quantum yield values of photosystem II (Φ_{PSII}) of the treatments, measured at 10 am, during the experimental period.



The asterisk represents the statistical difference ($p \leq 0.05$) on the evaluation day.

Figure 4 - Photochemical extinction coefficient values (*qP*) of the treatments, measured at 10 am, during the experimental period.

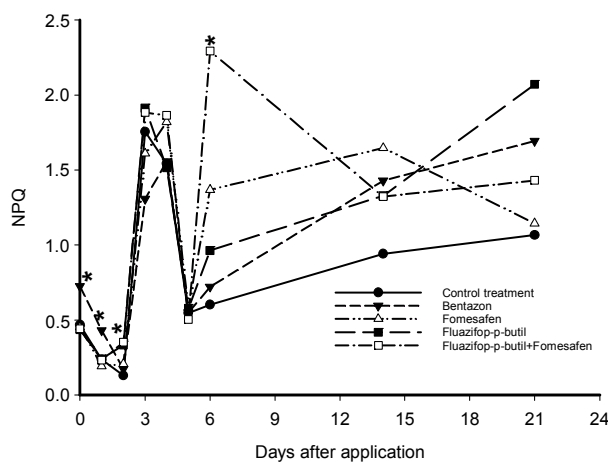
days. As for Φ_{PSII} , there was greater variation in its values, without result uniformity in the following measurements. Fomesafen presented significantly reduced values at 2 and 4 DAA, compared to other treatments, but with very close values. At 6 and 21 DAA, there was greater variation, and all herbicide treatments presented differences in relation to the control treatment that had been weeded at 6 DAA. Fluazifop-p-butyl differed from bentazon at 21 DAA, presenting reduced values in relation to it, but without differences in relation to the others (Figure 3). On the other hand, treatments with fomesafen, bentazon and fluazifop-p-butyl caused significant differences, compared to the control treatment with manual weeding, at 6, 14 and 21 DAA, respectively, for qP (Figure 4).

Φ_{PSII} is one of the most used variables to quantify the effective photochemical efficiency of PS II, through the percentage of light that is absorbed in chlorophyll molecules connected to PS II, which is effectively used in photochemical reactions, therefore depending on the PPFD at the time of the measurement (Roháček et al., 2008), which were quite variable between measurement days and even between readings on the same day. On the other hand, qP, although having a very similar definition, gives an indication of the proportion of PS II reactive centers capable of receiving electrons, which are less affected by PPFD (Maxwell and Johnson, 2000). The commercially available photosynthetic activity-inhibiting herbicides bind to the D1 protein of the PS II reaction center, and prevent the transfer of electrons that are in the PS II reaction center to the electron transport chain in chloroplasts (Pospíšil, 2009; Trebst and Draber, 2013). With this interruption, the percentage of light used in the photosynthetic process is reduced, and this is reflected in the marked decline of Φ_{PSII} and qP for bentazon at the beginning of determinations.

At 0 and 1 DAA, only bentazon had significantly higher NPQ values than those of the control treatment. Later, at 2 and 6 DAA, fluazifop-p-butyl, fomesafen and the herbicide mixture were also significantly higher than the values of the weed control treatment (Figure 5). The significant increase of NPQ soon after herbicide application indicates a stressful situation (Horton et al., 2005; Roháček et al., 2008), which, in this case, was caused by the reduction of the electron flow in photosystems caused by the herbicide. This was demonstrated by the decrease of Φ_{PSII} and qP (Figures 3 and 4). NPQ indicates the dissipation of excess energy in PS II, and occurs in almost all photosynthetic eukaryotes through mechanisms that help regulating and protecting the photosynthetic apparatus in environments where the absorption of light energy exceeds the photosynthetic use capacity of light (Baker, 2008). NPQ is a variable indicating the dissipation of non-photochemical energy in PS II by heat, mainly through the xanthophyll cycle, avoiding the production of ROS, which would cause degradation and loss of membrane integrity in thylakoids and photosystem activity; it is frequently increased by high PPFD values associated with stress,

such as by the action of herbicides on photosystems (Zivcak et al., 2015; Takahashi and Badger, 2011). Schreiber et al. (1994) reported that the relation between NPQ and excess PPFD occurs linearly, and that this linearity is also observed when NPQ is related to the production of zeaxanthin in the xanthophyll cycle of chloroplasts (Baker, 2008).

The fluctuations occurred in the variables presented in Figures 3, 4 and 5, obtained on light-adapted leaves (Φ_{PSII} , qP and NPQ), must have been due to the great variation of the environmental conditions at field level, especially PPFD and temperature, which can vary instantaneously and influence measures on light-adapted leaves, such as Φ_{PSII} , qP and NPQ ones. Other calculated variables, such as the qN non-photochemical extinction coefficient and the electron transport rate in photosystems, were not presented due to the greater variation of the obtained results.



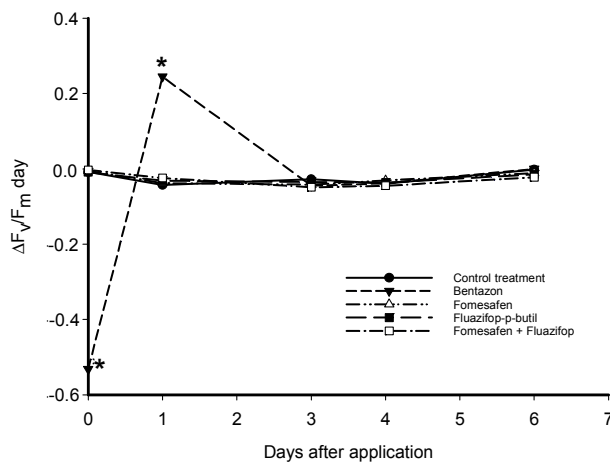
The asterisk represents the statistical difference ($p \leq 0.05$) on the evaluation day.

Figure 5 - Non-photochemical extinction coefficient values (NPQ) of the treatments, measured at 10 am, during the experimental period.

Pimentel (2014) comments that, under PPFD and temperature controlled conditions in growth chambers, it is possible to obtain great efficiency of the photosynthetic process. However, in a natural environment, plants often experience instant variations in PPFD, temperature and vapor pressure deficit in the air. These factors may lead to fluctuations in the fluorescence variables of chlorophyll *a* on light-adapted leaves, especially in ETR, since PPFD enters its calculation between one measure and another (De Bianchi et al., 2010).

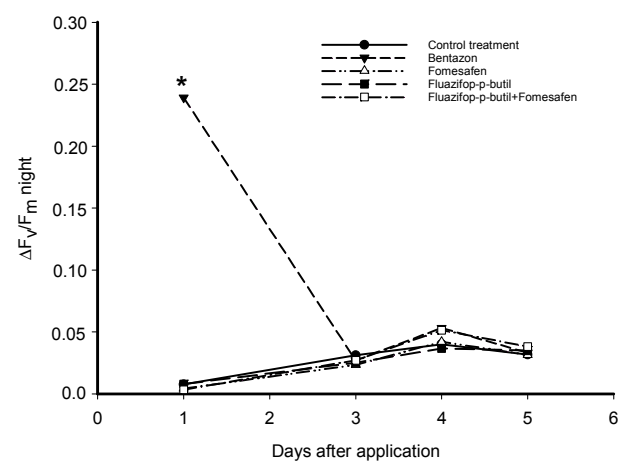
In order to evaluate the daily photoinhibition intensity associated to the herbicide effect, the difference between the F_v/F_m value measured at night and the one measured in the morning of the same day ($\Delta F_v/F_m$ of the day) was calculated; it is shown in Figure 6. In the morning, plants normally present greater photosynthetic activity than in the hours with higher PPFD (Pimentel, 2006), starting from the middle of the day, because environmental stresses may occur, due to high temperature or high transpiration associated to a higher PPFD at that specific moment; thus, the photosynthetic activity may be reduced, and plants may or may not express their photosynthetic potential, depending on the intensity of the stress associated with high PPFD (Roháček et al., 2008; Takahashi and Badger, 2011). Therefore, the lower the $\Delta F_v/F_m$ values of the day, the greater the photoinhibition associated with environmental or chemical stresses during that particular day (Pimentel, 2014). In this study, only the treatment with bentazon showed significant differences, with a reduction of mean $\Delta F_v/F_m$ value of the day to -0.532 at 0 DAA (Figure 6), and a positive value on the day after the application of the herbicide. The positive value at 1 DAA derived from a lower value found the morning after the day of bentazon application, compared to the night value at 1 DAA (Figure 6). The drastic reduction of the values measured at the beginning of the night period of 0 DAA is mainly due to the fast effect caused by the herbicide on PS II, with the interruption of the electron transport flow to the photosystems on the day of the application (Duke, 1990). Obstruction may cause oxidative stress due to the production of ROS under these photoinhibitory conditions, in which the photolysis of water keeps occurring, releasing electrons (Roháček et al., 2008). From the third DAA, it was no longer possible to verify significant differences in results between bentazon and other treatments, until the end of the evaluations (Figure 6). Therefore, from the third DAA, bentazon and other applied herbicides did not influence the photosynthetic activity of plants throughout the day in terms of F_v/F_m variable. Due to the lack of significance in the $\Delta F_v/F_m$ difference of the day, probably herbicides did not contribute to the aggravation of photoinhibition.

By subtracting the F_v/F_m values measured in the morning from the ones of the previous night ($\Delta F_v/F_m$ at night), it is possible to verify the capacity of recovering from environmental stresses associated with photoinhibition, during the entire night period (Figure 7), when the



The asterisk represents the statistical difference ($p \leq 0.05$) on the evaluation day.

Figure 6 - $\Delta F_v/F_m$ values of the day (F_v/F_m at night - F_v/F_m at dawn, on the same day) in bean plants submitted to the treatments.



The asterisk represents the statistical difference ($p \leq 0.05$) on the evaluation day.

Figure 7 - Values of $\Delta F_v/F_m$ at night (F_v/F_m at dawn - F_v/F_m of the previous night) in bean plants submitted to the treatments.

repair and recovery mechanisms of PS II activity are more effective than during the day, as there is no photoinhibition caused by excess PFD (Baker, 2008). Also in this case, only the bentazon treatment showed recovery capacity differences in relation to the control treatment, only at 1 DAA, with night $\Delta F_v/F_m$ values close to a 0.24 difference between the values measured the morning after the application of the herbicide, minus those from the night during which the herbicides were applied, at 0 DAA (Figure 7). The significant difference for the night $\Delta F_v/F_m$, which represents the night recovery capacity of the photoinhibition occurred the previous day, was only found at 1 DAA; this demonstrates the rapid recovery capacity of the photosystem activity, indicating rapid metabolism of the herbicide, by plants, soon after its application, avoiding a prolonged chemical stress. Besides the rapid recovery process, it is worth mentioning the overnight increase in the $\Delta F_v/F_m$ value, on the fourth day, for all treatments (Figure 7). The highest values obtained in this difference may be the result of a higher photoinhibition degree, to which plants were submitted at 3 DAA under high PFD; this reflected in a lower F_v/F_m value at the beginning of the night, compared to those found the following morning, after the night recovery from the photoinhibitory effect. It is important to note that in the third DAA, the highest PFD values were measured throughout the experimental period.

As for LSPC, there was no significant difference in the values of leaves collected at 0 DAA (Table 1), as well as after herbicide application, except for the fifth collection, performed at 14 DAA, where the treatment with fluazifop-p-butyl differed from the fluazifop + fomesafen mixture (Table 1). LSPC is proportional to the content of Rubisco, which corresponds to more than 50% of these soluble proteins (Pimentel, 2006). In case of stresses associated with high PFD, causing ROS formation, Rubisco can be degraded into chloroplasts, reducing more severely the photosynthetic potential of leaves (Baker, 2008; Raven, 2011). LSPC quantification, during the experimental period, shows that there was no negative influence of the herbicides on this variable and probably on the activity of Rubisco and CO_2 assimilation by the Calvin cycle, since there was no significance among the values found in treatments with herbicides in relation to the control treatment without herbicide application (Table 1). Thus, bentazon affected the photochemical activity in the photosystems until the first DAA (Figures 1, 2 and 3), but did not affect the assimilation potential of CO_2 by Rubisco in the Calvin cycle. The lack of herbicide interference in the LSPC of beans is beneficial and desirable, since the activity of Rubisco can probably be maintained; this would reflect in resuming the production of photoassimilated components as soon as the activity of the photosystems is resumed. This could ensure a good development of plants and embryos in the seeds, thus achieving better yields (Pimentel, 2006).

Table 1 - Leaf soluble protein content (LSPC, as mg of soluble protein g^{-1} of foliar fresh matter) of common beans, Carioca cultivar

Treatment	LSPC						
	Days after application						
	0	2	5	7	14	21	28
Control treatment	3.48 a	3.47 a	4.70 a	3.95 a	4.40 ab	4.24 a	4.02 a
Bentazon	3.45 a	3.54 a	4.66 a	3.44 a	4.01 ab	3.77 a	4.23 a
Fomesafen	3.37 a	4.17 a	4.86 a	3.75 a	4.27 ab	3.90 a	4.04 a
Fluazifop-p-butyl	3.50 a	3.72 a	4.59 a	4.05 a	3.75 b	4.12 a	3.96 a
Fluazifop-p-butyl + Fomesafen	3.49 a	3.91 a	4.33 a	3.74 a	4.93 a	3.81 a	4.30 a

Means followed by the same letter in the column do not differ by Tukey's test ($p \leq 0.05$).

In treatments with herbicides, the productivity of common beans was not affected by the applied doses (Table 2), indicating that, under these conditions, the herbicides were selective for the common bean crop, without affecting its productivity. Among production components, only the number of pods per plant differed significantly between the control treatment and the other ones, but this difference was not reflected in a higher productivity of the control treatment compared to the others (Table 2). Machado et al. (2006) evaluated the efficacy of the fomesafen, fluazifop-p-butyl and bentazon combination on weed management in common bean crops, and also observed selectivity and productivity maintenance that were similar to those of the control treatment without herbicides.

Table 2 - Number of pods per plant (NPP), number of seeds per pod (NSP), 100-grain weight (100GW) and grain productivity (GP) of common beans, Carioca cultivar, according to treatments

Treatment	NPP	NSP	100GW	GP
	(un.)	(un.)	(g)	(kg ha ⁻¹)
Control treatment	9.67 a	5.86 a	26.22 a	1920.5 a
Bentazon	7.62 b	5.65 a	25.84 a	2241 a
Fomesafen	7.45 b	5.32 a	26.32 a	1887.2 a
Fluazifop-p-butyl	7.0 b	5.45 a	25.84 a	1949.7 a
Fluazifop-p-butyl + Fomesafen	7.67 b	4.00 a	26.98 a	2095.5 a

Means followed by the same letter in the column do not differ by Tukey's test ($p \leq 0.05$).

In this study, it is possible to conclude that bentazon, acting on the photosystems, was the only one among the used herbicides used that caused reductions in F_v/F_m (Figure 1), Φ_{PSII} (Figure 2) and qP (Figure 3), and promoted NPQ increase (Figure 4), but only up to the first day after its application. F_v/F_m , measured on leaves adapted to the dark, was the most appropriate chlorophyll *a* fluorescence variable to evaluate the effects of applying herbicides on the photosynthetic apparatus of plants under field conditions with variable PPFD. The chlorophyll *a* fluorescence variables obtained on leaves adapted to light were strongly influenced by the environment, and were not recommended as indicators of herbicide effects in field studies. None of the herbicides caused LSPC alterations, and did not prove to contribute to the aggravation of photoinhibition throughout the day; grain yield was not affected, either.

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